1016-Plat
"Snorkeling" of the Charged Sidechain of a Transmembrane Peptide as Directly Observed by Double Electron-Electron Resonance Experiment
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While hydrophobic amino acids constitute the bulk of transmembrane protein domains, polar and even charged amino acids are not uncommon and often play significant roles in membrane protein function. Positioning a polar residue within the bilayer core is highly unfavorable thermodynamically; however, the free energy penalty could be minimized by ''stretching'' the side chain of the amino acid to bring the charged moiety closer to the bilayer surface while keeping the rest of the side chain inside the hydrophobic core. This biophysical phenomenon is known as ''snorkeling''. Here we report experimental observations of ''snorkeling'' for nitroxide-modified side-chains upon protonation, its dependence upon the location along the transmembrane peptide helix, and how this snorkeling is affected by the membrane electrostatic surface potential. pH sensitive spin labels, either IMTSL or IKMTSL (JPCB 2009, 113, 3453) were attached to two cysteine residues positioned equidistant from the center of the WALP peptide so that the primary sequence of each peptide is palindromic, thus, ensuring symmetric location of the labels with respect to the bilayer. The change in protonation states of the nitroxide was directly observed from EPR spectra. The distance between two nitroxide moieties was measured by Q-band double electron-electron resonance (DEER) experiment. Upon protonation, the distance between the two IMTSL probes increased compared to that of the neutral forms, by approximately 3 Å indicating displacements of the charged nitroxide sidechain towards the polar head region. The ''snorkeling'' of the label was observed to be depth dependent - no changes in the positioning of the sidechain upon protonation was observed for labels located within 10-8 Å from the center of the bilayer. Supported by NSF-0843632 to TIS.

Platform: Protein Structure and Conformation II

1017-Plat
Solution Conformation of the Unbound HIV-1 Protease Derived from Residual Dipolar Couplings Measured at Ambient and High-Pressure Conditions
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The flexibility of the glycine-rich flaps are known to be essential for the catalytic activity of the HIV-1 Protease but their exact conformations at the different stages of the enzymatic pathway remain yet to be determined. While hundreds of crystal structures of protease-inhibitor complexes have been solved, only a dozen of unbound protease structures have been reported to date. These structures reveal a large heterogeneity of flap conformations, ranging from closed, to semi-open and wide-open conformations. We used here NMR spectroscopy to determine the flap orientation of the unbound protease in solution. The conformation of the flaps was determined by comparing the measured residual dipolar couplings to reference crystal structures representing the closed, semi-open and wide-open states. The data clearly indicate that the apo protease adopts a closed conformation in solution, similar to an inhibitor-bound state.
We also compared the effect of high-pressure on the flap conformations of an apo and inhibitor-bound protease by measuring backbone residual dipolar couplings up to 1 kbar. A rigid-body motion model was derived to characterize the pressure-induced conformational changes of the flaps. While a minimal rearrangement of the flaps was observed in the case of the inhibitor-protease complex, we observed a significant pressure-induced rotation of the flaps in the case of the apo protease. These results highlight the intrinsic conformational flexibility of the unbound protease and offer a new perspective to understand the mechanism of ligand and inhibitor binding.

1018-Plat
Understanding Side Chain Conformational Variability in Proteins
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We present an advanced Monte Carlo method to generate protein side chain conformational ensembles to understand how ensemble properties like entropy can explain the improvement of a marginally performing enzyme. The Monte-Carlo Side Chain Entropy (MC-SCE) method combines the power of knowledge based rotamer libraries and a physics based all-atom force field (Amberff99SB+GB+HPMF) to describe side chain rotamer statistics under cooperative packing. The method was validated by comparing it's prediction with alternate side chain configurations seen in high-resolution X-ray crystal structures as well as $\chi 1$ J-coupling constants determined from solution NMR for a large set of proteins. Furthermore, it was observed that side chain entropy can be crucial in identifying native folds from a set of decoys. We have applied the MC-SCE method to understand the two-order improvement in catalytic activity of the designed KE07 enzyme by directed evolution. Currently, there is no rationale behind most of the mutations made and cause for catalytic efficiency that is observed. MC-SCE calculations on KE07 through the rounds of directed evolution suggest a systematic rigidification of the protein scaffold manifested in side chain entropy values and decorrelated motions with the catalytic residue quantified with mutual information. Finally, I will talk about how the MC-SCE analysis can be used to predict further mutations that improve biocatalytic performance.

1019-Plat
Navigating in the Protein Universe
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How are proteins related to each other? Which physicochemical considerations affect protein evolution, and how? Forming a global picture of the protein universe can help address these, and similar, questions. We study the evolutionary relationships among a representative set of 9,710 protein domains, taken from the SCOP database. We align all-against-all domains, searching for significantly-sized shared segments, referred to as motifs. These motifs have similar sequence and structure, and thus are indicative of evolutionary relationships among the proteins. The results are presented as a similarity network, in which edges connect domains that share a motif. Using reasonable similarity thresholds, the network manifests a large connected component, as well as many isolated 'islands', revealing the complex nature of protein space, which includes continuous and discrete regions. Overall SCOP domains of the all-alpha, all-beta and alpha+beta classes, in which alpha and beta elements do not mix, populate the discrete region, while alpha/beta domains, with their alternating alpha and beta elements, populate the continuous one. The network can be interpreted as a collection of evolutionary paths in protein space. The large amount of paths within the alpha/beta class suggests that it is particularly easy to add and delete motifs between them. Apparently, evolution took advantage of this property in order to design new proteins with novel functions. This is the first study that combines sequence and structural similarity between proteins within the context of a network to provide a bird's eye view of the protein universe. The network offers a natural way to organize and search in protein space; we provide tools to this end by integrating Cytoscape with PyMOL and other molecular viewers. They could be used to theorize about protein evolution, suggest evolutionary pathways between domains, and maybe also strategies for protein design.

1020-Plat
Protein Evolution across Fold Classes: A 3-α-Helix Bundle can Switch to β, α/β, and α+β Folds by Stepwise Mutation
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The mechanism for the evolution of new protein folds remains obscure. Recent examples of proteins with high levels of sequence identity but different folds have given new impetus to the hypothesis that proteins can evolve via stepwise mutation. Few examples of these metamorphic proteins are available, however, concealing the number of conformational alternatives accessible to a given fold. Here we show that stepwise mutation can switch one fold, a 3-α-helix bundle, to three radically different conformations: β-sheet, α/β/α-sandwich, and α/β-grasp. Specifically, we engineered three variants of the human serum albumin binding domain of protein G (G_A, 3-α-helix bundle) to have >50% sequence identity to three other proteins: a subdomain within outer surface protein A (OspA, β-sheet), a subdomain within maltose binding protein (MBP, α/β/α-sandwich), and the IgG-binding domain of protein G (G_B, α/β-grasp). Additional modifications to the sequences of these alternative folds yielded three fold pairs with high levels of sequence identity: 77% (G_A-OspA), 80% (G_A-MBP), and 98% (G_A-G_B). This network of switchable folds links all four major fold families, mainly α (3-α-helix bundle), mainly β (β-sheet), α/β (α/β/α-sandwich), and α+β (α/β-grasp), demonstrating that stepwise